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Prevalence of *A. actinomycetemcomitans* before and after wearing fixed orthodontic appliances. U. VAN DER VELDEN *, W.L. BURGER, A.J. VAN WINKELHOFF (Dept. of Periodontology & Oral microbiology,ACTA, Amsterdam, The Netherlands)

It has been suggested in the literature that subjects wearing fixed orthodontic appliances show a higher prevalence of *A. actinomycetemcomitans* than controls (Paolantonio et al. 1996). The purpose of the present study was to investigate longitudinally the occurence of *A. actinomycetemcomitans* in patients receiving fixed orthodontic appliances. The study population comprised 29 subjects who were scheduled to receive orthodontic bands at either 2 or 4 1st permanent molars. At these sites in each subject the following clinical parameters were evalated prior to banding and after wearing the bands for at least 8 months: Plaque (PI), Redness (R), Swelling (Sw), Probing pocket depth (PPD) and Bleeding on probing (BOP). In addition, a pooled subgingival sample was obtained from the experimental sites by means of paper points. The samples were cultured on TSBV for isolation and growth of *A. actinomycetemcomitans* . The patients received regular oral hygiene instructions during treatment. In total 25 subjects were available for the final evaluation. The mean values of the clinical parameters prior to and after wearing the bands were: PI 0.84- 0.52*, R 0.67-1.02*, Sw 0.49-0.95*, PPD 3.30-3.53 mm, BOP 0.95-1.10.The results for *A. actinomycetemcomitans* were as followes: prior to banding 5 subjects were pos.; 2 remained pos.; 2 became neg.;1 became pos. and 1 was lost for follow-up examination. In conclusion no increase in the occurence of *A. actinomycetemcomitans* due to orthodontic banding could be assessed.

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3-D Biofilm Patterns in Periodontal Diseases Defined by Confocal Microscopy. J. THOMAS*, K. LEVOS, R. WRATCHFORD, N. LUONG, R. CROUT. (West Virginia Univ., Morgantown, West Virginia).

Microbial biofilm patterns are increasingly important in adult periodontal disease (PD) definition. The purpose of this research was to expand previous measurements of the viable/non-viable signature of subgingival plaque in adult PD using target-specific vital fluorescent probes with Gram Stain (GS) enhanced by software for 3-D analysis (X, Y, and Z spatial arrangement). The Confocal Laser Scanning Microscope (CLSM) (Zeiss-510) used two laser sources: Argon (488 nm) and HeNe (Helium/Neon) (633 nm). The LIVE/DEAD BacLight Bacteria Viability Kit was used to assay contrasting fluorescent assays: SYTO 9 (viable) and Propidium Iodide (non-viable). Subgingival paper point samples from 15 healthy and 40 PD patients at two stages (Moderate and Severe) were analyzed. The 30-minute staining procedure rapidly distinguished 6 features including size, shape, membrane integrity (viable/non-viable), 3-D spatial arrangement, bioburden, and GS morphotype. Comparative results from the 20 Moderate and 20 Severe patients indicated that a 3-D microbial signature could be statistically correlated with disease severity. Disease progression shifted from low load, non-viable rods (75%) in healthy patients, to 50% coccobacillary (80% viable), in Moderate, to coccobacillary (80%) viable (95%) and closely packed in Severe disease. CLSM and vital probes with GS allows 6 simultaneous subgingival plaque characterizations in 3-D correlating with disease status and amplifies real-time assessment of intervention strategies.

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Relationship between Periodontal Pathogens and Clinical Parameters in Japanese Children. SUDA[1]*, R., KURIHARA[1], M., KURIHARA[1], C., SATO[1], T., HASEGAWA[1], K., LAI[2], C.H., LISTGARTEN[2], M.A. (Showa Univ.[1], Tokyo, JAPAN, University of Pennsylvania[2], PA, USA)

The purpose of this study was to determine prevalence of periodontal pathogens in Japanese schoolchildren and analyze the relationship of selected species to the following clinical parameters: Plaque Index (PI), probing depth (PD) and bleeding on probing (BOP) at the sampled sites. The mesial surface of one upper first molar was selected in each subject. Subgingival plaque samples from 29 children (8- to 10-year-olds) were collected and suspended in saline. Smears of plaque samples were prepared on glass slides and acetone-fixed. *Bacteroides forsythus (Bf), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Campylobacter rectus (Cr), Actinobacillus actinomycetemcomitans (Aa)* serotypes a and b were detected by an indirect immunofluorescence technique. The mean PI score (± SD) for the 29 subjects was 1.9 ± 0.7, PD was 2.2 ± 0.5 mm, and 69.0% of the 29 sites exhibited BOP. Of the sites with a PI of 1, 44% were BOP(+), while 80% of those with PI scores 2 and 3 were BOP(+)(p=0.09). Although all the children had gingivitis, probing depths were largely within the normal range. No *Pg* was found at any of the sampled sites. The prevalence of *PI* tended to be higher at BOP(+) sites than BOP(-) sites. The prevalence of *Pi, Aa*-a and *Aa*-b tended to be lower in 8-year-old children compared to 9- and 10-year-olds. However, the differences were not statistically significant. A negative correlation was observed between BOP and the levels of *Actinobacillus actinomycetemcomitans* (p<0.05). No correlation was observed between any bacterial species and PI or PD. Although periodontal pathogens are present in this young population, they do not correlate with gingivitis or periodontitis. However, their presence could predispose infected subjects to subsequent development of periodontitis. Supported by Ministry of Education, Science, Sports and Culture of Japan grant 9671984.

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Quantitative PCR using SYBR Green™ compared to nested fluorescent probes. B. DACE[1], A. PRABHU*, R.M. GLEASON, W.A. COULTER[1], C.E. SHELBURNE (3M Company, St. Paul, MN and [1]The Queens Univ. of Belfast, Belfast, Ireland).

The objective of this study was to evaluate two methods to quantitate PCR products of 16s rRNA genes of oral bacteria. We first used real-time PCR (R-PCR) using primers and nested fluorescent oligonucleotide probe (Taqman™) specific for B.forsythus 16s rRNA. The second method utilized a fluorescent DNA-binding dye (SYBR Green™) to measure PCR product accumulation. We compared the linear range and reproducibility of both methods using B.forsythus cultures. We found the SYBR Green™ assay was more sensitive than the Taqman™ procedure for quantitative detection of B.forsythus (2pg/µl vs. 20pg/µl, p<0.05). We observed several parameters that effect the results obtained by the methods included efficiency of the primers, size of the PCR product and background due to binding of SYBR Green™ to primers. High primer concentration resulted in primer-dimer, resulting in false positive signal and reducing the sensitivity of the SYBR Green™ system but had less effect on Taqman™. Differences in primer efficiency, as measured by the slope of the curves, resulted in variability of quantitation in both methods. We next compared the results obtained with 10 clinical plaque samples assayed by both the methods for quantitative detection of P.gingivalis, T.denticola and B.forsythus. Our results indicated that the clinical samples assayed with both methods gave similar results for all the bacteria measured (p<0.05), even though some samples were assayed one year apart. To check the ease of usage of SYBR Green™ assay, we examined 13 primer sets for bacteria taken directly from literature and amplified them using the conditions suggested by their authors. We observed that the SYBR Green™ assay worked for all the primer sets tested without any further optimization of the PCR conditions.

We conclude that the SYBR Green™ method is (1) simple, (2) readily applicable to many systems, (3) sensitive and specific depending on the specificity of the primers and (4) low level performance is sensitive to primer dimer accumulation at excessive primer concentrations.

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Periodontopathogens and GCF Levels of Granulocyte Elastase and IL-8 in EOP patients. L.J. JIN*, W.K. LEUNG, E.F. CORBET, X.X. MA, L.P. SAMARANAYAKE. (Faculty of Dentistry, The University of Hong Kong, Hong Kong).

This study aimed to determine the presence of subgingival periodontopathogens and their relations with the levels of granulocyte elastase activity (EA) and IL-8 in gingival crevicular fluid (GCF) in early-onset periodontitis (EOP) patients. Bleeding on probing and probing depth were measured using a Florida Probe®. GCF and subgingival plaque samples were collected from various clinical sites in 10 young Chinese adults, aged 19-24 yr., with untreated EOP. 10 age-matched, periodontally healthy subjects were used as controls. Granulocyte EA was analyzed with a specific substrate (pGluProVal-pNA) and the maximal rate of EA (MR-EA, mAbs/min/site) was calculated. IL-8 levels were determined by ELISA. Species-specific DNA probes were used to detect the presence of *A. actinomycetemcomitans* (*Aa*, ATCC 43718), *B. forsythus* (*Bf*, ATCC 43037), *P. gingivalis* (*Pg*, ATCC 33277), *P. intermedia* (*Pi*, ATCC 33563), and *T. denticola* (*Td*, ATCC 35405), with a sensitivity ≥ 10^3 cells/site. *Pg* and *Pi* were present in all EOP patients, *Bf* in 90%, *Td* in 80% and *Aa* in 40%. In healthy subjects, only *Pi* was found and only in 20% of the subjects. Co-infection with *Bf, Pg, Pi & Td* was detected in 80% of the patients and 31% of the sites sampled, of which 26% of the sites also harbored *Aa*. The prevalence of the co-infection with *Bf, Pg, Pi &Td* was higher at periodontitis sites (48%) than at healthy (17%) or gingivitis sites (17%) (p<0.05). Healthy subjects had higher mean of IL-8 concentrations (pg/µl) and lower mean MR-EA levels than the EOP patients (p<0.001). Healthy sites in control subjects also had a higher mean of IL-8 concentrations and lower mean of MR-EA levels than the healthy sites in patients (p<0.01). Within the 10 patients, higher MR-EA levels and lower IL-8 concentrations were found in periodontitis sites and in the sites co-infected with *Bf, Pg, Pi & Td* than in healthy sites or sites without the target species (p<0.01). Sites with the co-infection with *Bf, Pg, Pi & Td* showed significantly higher MR-EA levels and lower IL-8 concentrations than sites with similar gingival conditions and probing depths without the co-infection (p<0.01). This study indicates that an increased local inflammatory response is related to a co-infection of subgingival periodontopathogens in EOP patients. Combined tests of bacteria and host response may provide co-markers for a more thorough evaluation of EOP. Supported by Hong Kong RGC (HKU 7287/97M) and CRCG, HKU (337/254/0010).

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Effect of Scaling & Root Planing on IL-1β and ICTP Levels. R.J. ORINGER*, K. AL-SHAMMARI, W.A. ALDREDGE, V.J. IACONO, R. M. EBER, H-L. WANG, W.V. GIANNOBILE (S.U.N.Y. at Stony Brook, Stony Brook, NY, University of Michigan, Ann Arbor, MI).

Biochemical markers harvested from gingival crevicular fluid (GCF) may be useful for identifying and predicting disease progression and monitoring response to treatment. Interleukin 1β (IL-1β), a potent bone resorptive cytokine, and pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP), a host-derived breakdown product specific for bone resorption, have both been associated with periodontal tissue destruction. The aim of this study was to examine the effect of scaling & root planing (S/RP) on GCF levels of IL-1β and ICTP. Eighteen adult periodontitis subjects were monitored at 8 sites per subject at baseline (prior to S/RP), 1, 3, and 6-months. Four shallow (PD < 4mm) and 4 deep (PD ≥ 5mm) sites were monitored for both marker levels and clinical parameters. GCF was collected for 30 seconds on paper strips and levels of IL-1β and ICTP were determined using ELISA and RIA techniques, respectively. Clinical measurements included probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP). Deep sites exhibited significantly (p < 0.05) higher IL-1β and ICTP levels compared to shallow sites at all time intervals. ICTP levels among shallow sites were reduced at the six month visit (127.7 pg/site at baseline vs. 74.7 pg/site at 6 months, p=0.053). No significant differences were observed in marker levels at deep sites following treatment. These results suggest that while scaling and root planing lowered marker levels at sites exhibiting minimal periodontal breakdown, additional treatment may be required at sites with significant periodontal tissue destruction. (Supported by OraPharma, Inc., Warminster, PA)

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Relationship of GCF IgA, IgG and Beta-Glucuronidase to Changes in Periodontal Severity: R. E. SINGER*, J. GRBIC, A. BIESBROCK, C.H. YEH, I. LAMSTER, and N.P. LANG(The Procter & Gamble Co. Cinn Ohio; Columbia Univ., NY, NY; University of Bern, Bern, Switz)

The goal of this investigation was to determine the association between gingival crevicular fluid (GCF) IgA and IgG concentrations, as well as GCF Beta-glucuronidase levels with gingivitis, pocket depth (PD) and attachment level (AL) and changes in those clinical parameters in an adult population with gingivitis and mild/moderate periodontitis. Subjects (n=110, mean age = 46.7) were enrolled in this study and at baseline graded for gingivitis (mean GI = 1.28) and examined by manual probing for full mouth PD (mean = 2.89mm) and AL (mean = 2.86). Twelve GCF samples were taken from six maxillary posterior teeth from each of 99 subjects. Six months later subjects were re-examined for GI, PD, and AL, and GCF samples were collected as at the baseline from 93 subjects. For subjects completing all phases of the study comparison of the six month clinical indices to baseline demonstrated significant decreases (p<0.001) in mean PD and AL but no significant change in GI. During that interval there were significant (p<0.001) increases in mean GCF IgA and IgG concentrations and a significant decrease in GCF BG level. At the baseline mean GI scores were positively correlated to mean GCF BG and IgG (r=0.26 and 0.43), while mean PD and AL were negatively correlated (r=-0.33 and -0.31) to mean GCF IgA concentration. Baseline GCF levels of BG were positively correlated with the change in mean PD and AL (r=0.27 and 0.22) over six months and the mean incidence of sites with 2 mm increases in PD (r=0.21). Conversely, baseline concentrations of GCF IgA and IgG were inversely related to the change in mean PD (r=-0. 29 and -0.43) and to the incidence of sites with 2 mm increases in PD (r=-0.29 and -0.35) and AL (r=-0.23 and -0.43). The baseline IgA concentration also was positively correlated (r=0.22) with the incidence of sites with 2 mm decreases in PD. In sum, the relationships seen here between GCF IgA, IgG and BG levels and the changes in clinical measures of periodontal severity suggest these GCF parameters reflect distinct host response processes and might be useful correlates to clinical outcomes.

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Elevated levels of sVCAM-1 in the GCF of patients with periodontal disease. E. HANNIGAN[1]*, LOUIS A. BUCKLEY[2], D.P. O'CONNELL[1] ([1]Dept. of Pharmacology, [2] University Dental Hospital, University College Cork, Ireland.)

Vascular cell adhesion molecule-1 (VCAM-1) is a cell surface protein involved in the adhesive interactions between cells. It is upregulated following activation during inflammatory responses, mediating both cell migration and activation. It has been suggested that this molecule may act as a site-specific marker of periodontal disease activity. The aim of this study was to determine the levels of soluble VCAM-1 (sVCAM-1) in the gingival crevicular fluid (GCF) of clinically healthy subjects and subjects with adult periodontal disease. GCF was collected from a healthy, a gingivitis and a periodontitis site in twenty-nine subjects with periodontitits and from a healthy site in twenty-two subjects without periodontitis. The volume of GCF was measured and the concentration of sVCAM-1 was determined for each sample using an enzyme-linked immunosorbent assay. There were statistically significant differences between the concentrations of sVCAM-1 in gingivitis and periodontitis sites compared with healthy sites (p<0.05). sVCAM-1 was significantly elevated in the healthy sites of periodontitis subjects compared with clinically healthy subjects. These results show that there are elevated levels of sVCAM-1 in the healthy, gingivitis and periodontitis sites of the disease group when compared to the controls and may suggest that elevated levels of sVCAM-1 can act as a site-specific biomarker of periodontal disease activity. This study was supported by the Health Research Board and the President's Research Fund, U.C.C., Ireland.